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by

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16. ABSTRACT Drinking Water obtained from surface sources contains a very large variety of organic chemicals. The total organic carbon present in the source water is made up of both natural and man-made chemicals. In most instances natural organic material predominates and is largely made up of humic and fulvic acids. The introduction of chlorine into drinking water results in the formation of a variety of by-products including the trihalomethanes, haloacetonitriles, halogenated aldehyde and halogenated ketone derivatives. Representatives of these classes of chemicals have been shown to be mutagenic and/or carcinogenic. More recent studies have shown that similar chemicals are formed upon direct administration of chlorine solutions to rats. Hypochlorite and monochloramine (a common alternative disinfectant to chlorine) have been shown to be capable of increasing the percent of structurally abnormal spermheads in mice at low doses (4 mg/kg per day for five days). Chlorine dioxide, a proposed alternative disinfectant, has been shown to produce decreases in plasma thyroxine levels at exposures of 100 mg ClO₂/L of drinking water. It is unlikely that these effects can be attributed to direct effects of disinfectants since they are all extremely reactive molecules and would react freely with the great excess of organic material present in the gastrointestinal tract. It is more likely that these effects can be attributed to reaction products such as those which have been identified in drinking water and/or the stomach contents of experimental animals. Therefore, these data suggest that chemical interactions between a group of chemicals that have been generally regarded as safe (the disinfectants) and other chemicals of a low level of intrinsic toxicity (humic acids, stomach contents) produce potentially hazardous products.		
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INTRODUCTION

A chemical is thought to modify the toxic effects of a second chemical by one of 4 general mechanisms. Most often considered are pharmacological interactions, or those situations in which there is a commonality in the receptor through which the toxic chemicals exert their effects. The net result of the interaction depends upon the relative affinity of the chemicals (or their active products) and whether the chemicals act as agonists or antagonists. The second type of interaction is that in which a chemical modifies the uptake, distribution, metabolism or excretion of a toxic chemical. Third are interactions that depend upon the activation or inhibition of physiological systems that compensate for or amplify the effects of a second chemical. Finally, there are the cases where one chemical actually reacts with a second chemical within the body to produce a more or less toxic group of chemicals.

The present paper deals with an example of the 4th type of interaction, that of chemical reaction. Disinfection of drinking water utilizes reactive chemicals,

usually oxidizing agents, that react with biochemical components of bacteria, viruses and parasites to kill these infectious agents. The effectiveness of this approach has been demonstrated by the marked decrease in waterborne infectious disease in this country since the early 20th Century. However, it is becoming increasingly apparent that disinfectants also react with organic materials present in water or in the gastrointestinal tract to produce potentially toxic by-products. This was first realized when it was found that chlorination of drinking water gave rise to trihalomethanes (Bellar and Lichtenberg, 1974; Rook, 1974). This area has become increasingly complex in the last 2-3 years as diversity of substrates that are available for reaction has become recognized. As might be suspected the diversity of toxicological properties associated with these reactions is also growing. For the purposes of this paper, however, only reactions of chlorine to produce chemicals that possess carcinogenic and mutagenic properties will be considered.

Reaction of Chlorine with Organic Chemicals in Drinking Water

Meier et al. (1983) demonstrated that treatment of humic acid with chlorine produced direct acting mutagens in the standard plate assay of Ames (1975) utilizing Salmonella tester strains. In Table 1 the effect of varying pH on mutagen formation is compared. Mutagen formation and chlorine substitution, indicated by formation of total organic halogen (TOX), is very much favored when the pH is allowed to drift to acid pH following addition of chlorine. Although there is still considerable TOX formed at alkaline pH, the mutagenic activity is sharply reduced. This circumstance is at least partially attributable to the fact that mutagens formed at acid pH are very alkaline labile (Meier et al., 1983). It is

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notable that trihalomethane formation is favored at alkaline pH and they would not be detected under the test conditions used because of their volatility.

Mutagenic activity formed is proportional to the degree of chlorine substitution as determined with total organic halogen analyses at chlorine to carbon molar ratios between 0 to 1.0. It is difficult to determine the extent to which this is only a fortuitous relationship since the relative importance of oxidation reactions versus chlorine substitution reactions of HOCl/OCl^- in the production of mutagenic activity has not been established. Interpretation is very clouded by the fact that mutagens formed are very alkaline labile.

More than 40 compounds have been tentatively identified in these reaction mixtures (Coleman et al., 1984). They fall into several classes, the trihalomethanes, chlorinated alkanes, alkenes, aldehydes, ketones, acids, nitriles and aromatics (Table 2). Among these chemicals are a number of established carcinogens and mutagens and others which have been demonstrated to possess such properties in our own Laboratory.

Despite the fact that a number of mutagenic chemicals have been identified, to this point only about 1.5% of the total mutagenic activity can be accounted for by chemicals for which both quantitative analyses and mutagenic characterization has been possible. Admittedly, this is somewhat of an artifact of the lack of appropriate standards and the time lag associated with getting these chemicals synthesized and tested. Nevertheless this figure does provide some estimation of the complexity of the overall problem.

Adding to this complexity is that the formation of mutagenic activity and the identity of products can be markedly changed by incorporating trace quantities of Br^- into the reaction mixture. As Br^- is increased to a molar ratio of 0.1 to the ^{amount of} chlorine added there is an approximate doubling of the mutagenic activity (Meier, unpublished observations). As would be predicted from the well-known ability of HOCl to activate Br^- to HOBr , bromination reactions begin to predominate. As can be seen in Table 3 brominated compounds become very evident at ratios of Br^- to HOCl of 0.01 and progressively increase until completely brominated compounds predominate at ratios above 0.1.

By-Products of Chlorine Produced in vivo

Drinking water in the U.S. is treated to a residual. This means that water consumed by the public would usually contain between 0.5 and 2 mg/L of disinfectant. It is obvious that the variety of potential substrates for chlorine available within the gastrointestinal tract adds another dimension to this problem. The question naturally arises "can interactions of chlorine reactions with substrates present in the G-I Tract result in products with carcinogenic and mutagenic properties?"

Experiments have been sponsored by our Laboratory that show that OCl^- and ClNH_2 administered orally increase spermhead abnormalities in B6C3/F₁ mice (Meier et al., 1983). An increased percentage of abnormal sperm was seen at 3 weeks following dosing, but not at 1 and 5 weeks, consistent with an effect at the spermatocyte stage of spermatogenesis. HOCl was without effect in this system. It is considered unlikely that OCl^- is capable of reaching the testis because of

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its reactivity. Therefore, it is felt that these data are evidence for the formation of highly mutagenic chemical(s) in the gastrointestinal tract. It should be noted that conditions of pH responsible for the formation of mutagens in vivo are the opposite of what was observed in reactions with humic acid. The fact that the same result was observed in two experiments with OCl^- (pH 8.5) and with ClNH_2 (pH 9) indicates a degree of consistency in the results.

To document the extent to which chlorination by-products can be formed in the gastrointestinal tract, Mink et al. (1983), intubated rats with relatively high doses of OCl^- (ca. 150 mg/Kg) and observed the appearance of by-products in the stomach contents and plasma. Table 4 lists the products identified by GC/MS when animals were treated with a chlorine solution containing Br^- . Within the stomach contents many of the same products seen in the reactions with humic acids were identified, but could not be meaningfully quantitated. Primarily we saw halomethanes, haloacetonitriles and halogenated acetic acids were observed.

To what extent can the spermhead abnormalities be accounted for by these products? Not at all, it would appear. Topham (1980) demonstrated that chloroform is negative in this bioassay. Our laboratory has tested the haloacetonitriles identified in these experiments in the mouse spermhead assay at much higher doses than would be expected to be formed in the G-I tract and found they were negative as well (Bull et al., unpubl. results). Although we have not yet tested this possibility it would seem highly unlikely that these effects could be attributed to the highly polar haloacetic acid products formed both because of a lack of intrinsic activities in other mutagenesis test systems and on pharmacokinetic grounds.

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However, it is clear that the haloacetonitriles do possess significant biological properties (Table 5). Two haloacetonitriles, dichloroacetonitrile and bromo- chloroacetonitrile, are mutagens in Salmonella. In addition, the dibromo-, the bromochloro-, the trichloro- and the monochloro- acetonitriles act as tumor initiators in the mouse skin (Bull et. al., unpubl. results). The ability of these same 4 compounds, to produce lung adenomas has also been demonstrated in Strain A mice with oral administration of 10 mg/kg 3 times weekly for 8 weeks.

Conclusions

The major conclusion of this work is that toxic properties associated with reactive chemicals such as the drinking water disinfectants must be viewed as being potentially quite diverse. It is clear that a diversity of mutagenic and carcinogenic chemicals and in addition to the trihalomethanes are formed both in drinking water and in vivo through reactions of chlorine with endogenous substrates. Evaluation of the overall toxic effects of chlorine must deal with the diversity of products that can be formed and the fact that these products have their own intrinsic toxicological properties. To complicate the situation, it is also quite clear that the nature of the products formed will vary with the substrates available in the diet. Amino acids have been shown as substrates that can give rise to haloacetonitriles (Bieber and Trehy, 1982). They may also give rise to a group of organic chloramines (Scully et al., 1983), an example of which, N-chloropiperidine, is extremely potent in producing spermhead abnormalities in mice.

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In summary, reaction of chlorine in biological systems will necessarily give rise to a very complex mixture of organic chemicals. It remains to be determined whether the toxic chemicals that arise from reactions of chlorine are produced in sufficient quantities to present a meaningful hazard to human health. However, it should be recognized that by-products of chlorination are the most common and plentiful organic chemicals identified in drinking water. Their levels outstrip the levels of industrial chemicals by 1-2 orders of magnitude in finished waters taken from surface sources. Finally, it must be recognized that other potential disinfectants are also reactive chemicals (chloramine, chlorine dioxide and ozone). Therefore, analogous problems will also arise from their use. The only resolution of this problem is a systematic study of the reactions of these chemicals, identification of the major by-products and characterization of their toxicological properties. Once this is established the toxicological properties of these extremely complex mixtures could be meaningfully, usefully studied.

The research described in this article has been reviewed by the Health Effects Research Laboratory and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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TABLE 1. COMPARISON OF THE pH DEPENDENCE OF MUTAGEN
FORMATION UPON TREATMENT OF HUMIC ACID WITH
CHLORINE (HOCl/OCl)

NO.	SAMPLE	TOX	MUTAGEN ACTIVITY ^a	
			TA98	TA100
A	CHLORINATED HUMIC (pH: 7.0 - 2.8)	414	339 $\pm$ 29 (100)	1696 $\pm$ 148 (100)
B	CHLORINATED HUMIC (pH: 7.0 - 6.5)	263	62 $\pm$ 10 (18)	367 $\pm$ 34 (22)
C.	CHLORINATED HUMIC (pH: 11.5 - 6.5)	280	N.S. ^b (15)	490 $\pm$ 33 (29)
D	NON-CHLORINATED	0.3	N.S. ^b (15)	N.S. ^b (15)

^a NET REVERTANTS/ML OF SAMPLE, CALCULATED FROM THE LINEAR PORTION OF DOSE RESPONSE CURVE. NUMBERS IN PARENTHESES INDICATE THE PERCENT OF ACTIVITY IN SAMPLE A.

^b N.S. = NOT SIGNIFICANT (I.E., LESS THAN 2-FOLD ABOVE BACKGROUND) FOR THIS EXPERIMENT. NEGATIVE CONTROL VALUES WERE 21 REVERTANTS/PLATE FOR TA98 AND 98 REVERTANTS/PLATE FOR TA100; THE HIGHEST DOSE LEVEL WAS 0.4 ML. THEREFORE, A N.S. RESPONSE WOULD BE LESS THAN 52 REVERTANTS/ML FOR TA98 AND LESS THAN 245 FOR TA100.

Table 2. TENTATIVE IDENTIFICATION OF MAJOR CONSTITUENTS
OF CHLORINATED HUMIC ACID^a

<u>TRIHALOMETHANES</u>	<u>KETONES (CONT.)</u>
**CHLOROFORM	1,3-DICHLORO-2-BUTANONE
	1,1-DICHLORO-2-BUTANONE
	3,3-DICHLORO-2-BUTANONE
<u>ACIDS</u>	1-CHLORO-3-BUTEN-2-ONE
DICHLOROACETIC ACID	3-CHLORO-3-BUTEN-2-ONE
TRICHLOROACETIC ACID	DICHLORO-3-BUTEN-2-ONE
	TRICHLORO-3-BUTEN-2-ONE
<u>ALDEHYDES</u>	TETRACHLORO-3-BUTEN-2-ONE
DICHLOROACETALDEHYDE	PENTACHLORO-3-BUTEN-2-ONE
TRICHLOROACETALDEHYDE	TRICHLOROCYCLOPENTENEDIONE
DICHLOROPROPANAL	
TRICHLOROPROPANAL	<u>NITRILES</u>
*2-CHLOROPROPENAL	**CHLOROACETONITRILE
2,3-DICHLOROPROPENAL	*DICHLOROACETONITRILE
*3,3-DICHLOROPROPENAL	*TRICHLOROACETONITRILE
*2,3,3-TRICHLOROPROPENAL	DICHLOROPROPANENITRILE
TRICHLOROBUTANAL	DICHLOROPROPENENITRILE
DICHLOROBUTENAL	TRICHLOROPROPENENITRILE
<u>KETONES</u>	
1-CHLORO-2-PROPANONE	<u>AROMATICS</u>
*1,1-DICHLORO-2-PROPANONE	**2,4,6-TRICHLOROPHENOL
*1,3-DICHLORO-2-PROPANONE	TRICHLORODIHYDROXY BENZENE
*1,1,1-TRICHLORO-2-PROPANONE	TETRACHLOROTHIOPHENE
1,1,3-TRICHLORO-2-PROPANONE	
1,1,1,3-TETRACHLORO-2-PROPANONE	<u>ALKANES AND ALKENES</u>
1,1,3,3-TETRACHLORO-2-PROPANONE	**HEXACHLOROETHANE
*PENTACHLOROPROPANONE	*PENTACHLOROPROPENE
3-CHLORO-2-BUTANONE	TETRACHLOROCYCLOPROPENE
1,1,1-TRICHLORO-2-BUTANONE	HEXACHLOROCYCLOPENTADIENE
1,1,3-TRICHLORO-2-BUTANONE	

^a DATA OBTAINED FROM COLEMAN ET. AL. (1984).

* CHEMICALS IDENTIFIED AS MUTAGENS

** CHEMICALS IDENTIFIED AS CARCINOGENS

TABLE 3. COMPOUNDS IDENTIFIED^a IN METHYLENE CHLORIDE EXTRACTS
OF HUMIC ACIDS TREATED WITH CHLORINE^b WITH VARYING
CONCENTRATIONS OF BROMIDE (BR⁻)^c

COMPOUNDS	MOLAR RATIO OF BR ⁻ /C WHERE COMPOUND CONCENTRATION IS HIGHEST ^d
1,1,1-TRICHLORO-2-PROPANONE ^e	0
PENTACHLORO-2-PROPANONE ^e	0
CHLOROFORM	0.01
TRICHLOROACETONITRILE	0.01
BROMODICHLOROMETHANE	0.01
DICHLOROACETONITRILE	0.01
1,1-DICHLORO-2-PROPANONE	0.01
BROMOCHLOROMETHANE	0.05
BROMOCHLOROACETONITRILE	0.1
DIBROMOCHLOROMETHANE	0.1
BROMOCHLOROACETONITRILE	0.1
1,1,1-BROMODICHLORO-2-PROPANONE ^e	0.1
1,1,1-DIBROMOCHLORO-2-PROPANONE ^e	0.1
BROMOFORM	0.5, 1
TETRABROMOMETHANE	1
1,1,1-TRIBROMO-2-PROPANONE ^e	1

^a 60M x 0.25 MM I.D. DB1 FUSED SILICA COLUMN

^b Cl:C MOLAR RATIO 1:1

^c DATA OBTAINED FROM COLEMAN ET AL. (1984)

^d APPLICABLE ONLY TO RATIOS TESTED, CONCENTRATION IS RIC AREA

^e STANDARD NOT AVAILABLE, IDENTIFIED BY MANUAL INTERPRETATION OF MASS SPECTRUM

TABLE 4. HALOGENATED PRODUCTS OBSERVED IN THE RAT STOMACH AND BLOOD PLASMA FOLLOWING ACUTE TREATMENT WITH CHLORINE AND KBr^a

COMPOUND	STOMACH CONTENT	PLASMA
CHLOROFORM	+ ^b	-
BROMOFORM	+	+
BROMODICHLOROMETHANE	+	-
DIBROMOCHLOROMETHANE	+	+
DICHLOROACETONITRILE	+	-
TRICHLOROACETONITRILE	-	-
DIBROMOACETONITRILE	+	-
DICHLOROACETIC ACID	+	-
DICHLOROACETIC ACID	+	-
TRICHLOROACETIC ACID	+	-
DIBROMOACETIC ACID	+	-
DIBROMOMETHANE	+	-

^a TREATMENT INVOLVED GAVAGE WITH TOTAL DOSE OF 48 mg Cl (as NaOCl) and 32 mg Br⁻(as KBr). ANALYSES CONDUCTED 1 h AFTER DOSING.

^b POSITIVE SIGN (+) INDICATES IDENTIFICATION OF THE INDICATED COMPOUND BY GC/MS. NEGATIVE SIGN (-) INDICATES COMPOUNDS WERE BELOW DETECTION LIMIT.

TABLE 5. ACTIVITY OF HALOACETONITRILES IN MUTAGENESIS
AND CARCINOGENESIS SCREENING PROCEDURES

	SALMONELLA ^a	SCE INDUCTION	TUMOR INITIATION MOUSE SKIN ^b	MOUSE LUNG ADENOMAS
CHLOROACETONITRILE	-d	+	+	++
DICHLOROACETONITRILE	++	+	+	+
TRICHLOROACETONITRILE	--	++	+	+
DIBROMOACETONITRILE	--	+++	+++	+
BROMOCHLOROACETONITRILE	+++	+++	++	+++

^a MEIER ET AL., PERSONAL COMMUNICATION.

^b BULL ET AL., (1982^b) TOXICOLOGIST 2:326.

^c BULL ET AL., (1983) UNREPORTED DATA.

^d INDICATION OF NEGATIVE (-) OR RELATIVE DEGREE OF POSITIVE RESPONSE (+).